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Single-Ring GroEL: An Expanded View

A remarkable structure of an 86 kDa substrate encapsulated in a single-ring GroEL/GroES chaperonin complex is revealed by cryo-electron microscopy in this issue of *Structure* (Chen et al., 2006). Surprisingly, the protein-folding chamber is 80% larger than that of the double-ring GroEL/ES structure.

Many newly synthesized proteins in both eukaryotes and prokaryotes depend on the type I chaperonins for proper folding. The *E. coli* GroE system comprises two heptameric homo-oligomeric GroEL rings associated back-to-back, defining chambers at both ends, and the heptameric cochaperone GroES. ATP binding to one ring triggers the capping of GroES onto this "*cis*" ring, sequestering a protein substrate and facilitating its proper folding (Figure 1A). After ATP hydrolysis by the *cis* ring, binding of ATP to the opposite "*trans*" ring prompts the release of GroES and substrate. Cycles of entrapment and folding are controlled by positive cooperativity within rings and negative cooperativity between rings (Yifrach and Horovitz, 1994).

Eukaryotic Hsp60, found in mitochondria and chloroplasts, shares 51% sequence identity with GroEL, and it has been assumed that the two systems are mechanistically similar. Indeed, it has been shown that Hsp60 and its cochaperone Hsp10 can substitute for GroEL/ES in vivo, when expressed in *E. coli* cells deficient in GroEL and GroES (Nielsen et al., 1999). One major difference between the two complexes is in the stability of the oligomers. The Hsp60/10 system is less stable, apparently operating as a single-ring entity (Viitanen et al., 1992) or as an equilibrium mixture of monomers and double-ring (DR) structures (Levy-Rimler et al., 2001).

A model for characterizing single-ring (SR) chaperonins is the GroEL mutant protein "SR1," which contains four amino acid substitutions that eradicate inter-ring contacts. SR1 is able to bind substrate and GroES, but is unable to release them (Weissman et al., 1996). However, full chaperonin functionality both in vivo and in vitro is restored by a variety of additional single mutations in SR1 (Sun et al., 2003). Thus, folding of functionally active obligate substrates by only a single ring of GroEL/ES is apparently possible.

A first view of an SR GroEL/GroES complex together with substrate cargo inside is provided by the cryo-electron microscopy (EM) study of Chen et al. (2006). The result is surprising for two important reasons: (1) The substrate is an 86 kDa heterodimer, too large to fit into the "standard" GroEL/ES Anfinsen cage, which has a mass cutoff for unfolded proteins of about 57 kDa. (2) An "expanded" SR GroEL/ES conformation is described, including never-before observed severe deformations, resulting in the cage volume increasing by 80%.

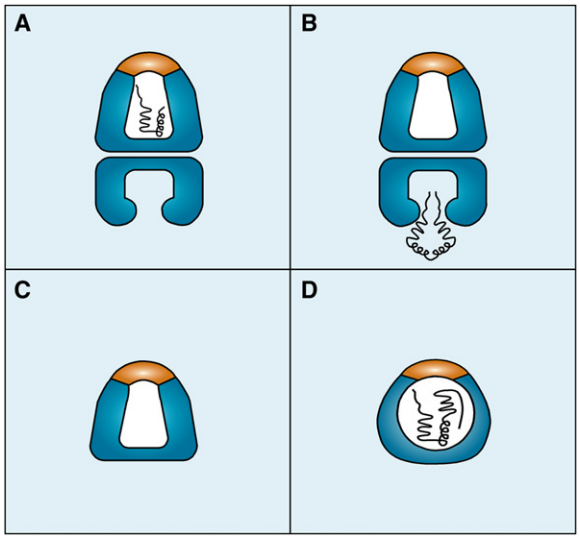

Figure 1. GroEL and GroES Create a Chamber Termed the "Anfinsen Cage"

Substrate encapsulation mechanisms (GroEL, blue; GroES, orange). (A) Substrate (<57 kDa) binds to the *cis* ring (i.e., the same ring that binds GroES) and is trapped by GroES. (B) Large substrate (>57 kDa) binds opposite to the *cis* ring, requiring GroES binding for release of substrate. (C) "Standard" single-ring conformation of SR398-ES. (D) "Expanded" single-ring conformation of SR398-ES, with large substrate entrapped.

The first point highlights the problem that despite the apparent mass cutoff of 57 kDa, some proteins that exceed it are dependent on both GroEL and GroES for proper folding. For example, among yeast proteins that are imported into isolated intact mitochondria, some very large substrates were found that are obligate substrates of both Hsp60 and Hsp10, including the 82 kDa aconitase (Dubaquie et al., 1998). The suggestion that *cis* GroES binding could promote release of large substrates bound to the opposite *trans* ring (Figure 1B) (Inbar and Horovitz, 1997) was demonstrated for folding of aconitase by GroEL/ES in vitro. The mechanism, which was elucidated in a set of elegant experiments (Chaudhuri et al., 2001; Farr et al., 2003), is obviously not available for SR GroEL/ES. So, if the Hsp60/10 system functions as a single-ring entity, as some researchers propose, how *do* SR chaperonins accommodate large substrates?

The current cryo-EM study is of an SR GroEL mutant protein defective in ATP hydrolysis, SR398. Thus, a stable population of particles encapsulating large substrates was obtained. Classification of images shows that even in the *absence* of substrate, there are two distinct populations of SR398-ES particles: 72% of them are in the "standard" conformation (i.e., similar to the *cis* conformation in the DR system) (Figure 1C), and 28% are in the "expanded" conformation. When substrate is added, the population of expanded structures soars to 80% (Figure 1D). Significantly, only the expanded form showed density corresponding to substrate in the cavity.

An interesting question is whether this expanded state is reversible; if substrate were removed, would the majority of particles return to the standard conformation?

The expanded structure is highly deformed from the standard state, but the relatively modest resolution of the reconstructions (12–16 Å), due to unresolved heterogeneity in the dataset, means that interpretation of what exactly happens to the GroEL domains is unclear, although the apical and intermediate domains appear to expand outwards, and equatorial domains slide inwards. Until now, conformational changes due to nucleotide and GroES binding were characterized as rigid-body movements (albeit large ones) of the intermediate and apical domains. This expanded state shows that the GroEL molecule must be much more flexible than previously thought. Characterization of this conformation in terms of tertiary structure will require higher resolution cryo-EM reconstructions.

Another obvious question is whether such an expanded state exists in the double ring system. Trypsin digestion experiments (Song et al., 2003) suggest that the heterodimeric substrate used by Chen et al. is protected not only by SR GroEL/ES, but also by the DR system. The expanded state would seem unlikely to occur in the DR system, since all inter-ring contacts would be disrupted. A cryo-EM study of the substrate trapped in the DR system would provide definitive evidence, but it is also obvious that this is a much more difficult task, due to much higher heterogeneity in the population of particles. It would be useful to demonstrate expanded-state *cis* encapsulation using a large *monomeric* substrate, in order to simplify interpretation.

An important point to remember is that the 86 kDa heterodimeric substrate chosen for this study might be partially folded inside the GroEL cavity, since it is a stable folding intermediate (Wynn et al., 1998). Thus, its volume inside the cavity would be less than for an unfolded substrate of similar mass.

Finally, a very difficult problem in cryo-EM reconstruction methods is to differentiate between (1) views of different orientations of the same structure in a homogeneous population and (2) actual structural heterogeneity in a particle population. In this study, the authors knew from the outset that heterogeneity must exist, since some particles would carry substrate and others would not, even without the expectation of two different conformations. They successfully sorted out this mess by starting with initial references that were identical except for the addition of random noise, and continuing with multiple-reference alignment. This appeared to have been enough to allow for separation of the mixed images into more homogeneous subgroups. It is an intriguing and simple tool, and hopefully it can be used generally in other cases of heterogeneous populations of conformations.

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RecA Assembly, One Molecule at a Time

Several recent papers have applied optical methods to directly visualize the assembly of individual RecA and Rad51 filaments on DNA. The hope is that application of such methods will shed light on the many mysteries that still surround how these remarkable filaments function in genetic recombination.

Although the bacterial RecA protein has been actively studied for almost 30 years with techniques including X-ray crystallography, electron microscopy, spectroscopy, biochemistry and genetics, it is fair to say that